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By.
B.F. Lutman.

Studies on Club-Root

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STUDIES ON CLUB-ROOT

I. THE RELATION OF PLASMODIOPHORA BRASSICAE TO ITS HOST AND THE STRUCTURE AND GROWTH OF ITS PLASMODIUM

By B. F. Lutman

SUMMARY

1. The swelling on the roots of cabbages and other cruciferous plants is due to the presence in the cells of these plants of a myxomycete, *Plasmodiophora Brassicae*.

2. This parasite makes its entrance into the root either through the epidermis or root hairs. Once within the plant it spreads from cell to cell either by actually penetrating the walls or through host cell divisions, carrying it into the daughter cells. It is found particularly in the cortex, but always makes its way into some cells of the central cylinder. The spread is largely among the cells of the primary cortex, but the fungus also seems to be able to infect those of later origin.

3. The infected host cells hypertrophy, but nuclear and cell division is not seriously interfered with in the earlier stages. In advanced cases both processes are entirely suspended and the nucleus undergoes degeneration. This process is marked by the formation of numerous nucleoli and by the breaking up of the linin and chromatin organization into irregular strands and clumps. The host starch disappears during the growth of the plasmodium, but the cells containing the myxomycete spores have little or none thereof.

4. The plasmodium of the fungus is clear and almost transparent at first, the outlines being difficult to distinguish as they blend with the protoplasm of the host. The plasmodia may increase in numbers by a self-fragmentation of pseudodia. The Plasmodiophora nuclei enclose a large karyosome or central body but are otherwise almost structureless.

5. Nuclear divisions in the plasmodium are of two types:—vegetative and reduction. The vegetative divisions are peculiar in that a spireme is not formed. The granules which seem to enclose the central body form a plate around it. These granules, and often the remains of the central body, simultaneously divide. The number of granules could not be determined. The reduction division is one of those preceding spore-formation, probably the first. The central body may break up by fragmenting into eight granules which later disappear on the spireme, or it may lie intact on one of the strands and gradually be used up. The latter is more frequently seen.

6. Spore formation and the segmentation of the protoplasm is brought about by vacuoles of various sizes separating the masses of

cytoplasm which have collected around the nuclei. No progressive cleavage such as is to be found in other myxomycetes is to be seen. Spores have been observed pinching into two after being thus formed.

7. No further nuclear divisions occur in the rounded up spores

8. The reduction divisions are apparently those preceding spore-formation and the reduced number of chromosomes is eight. The fusion of nuclei in the plasmodium must occur at some previous but as yet undetermined stage in the life-history of the Plasmodiophora.

INTRODUCTION

The cause of the abnormal swellings on the roots of cabbage and other cruciferous plants, known in various countries as "clubbing," "club-root," "finger and toe disease," "kohlhernie" or "maladie digitoire," was determined by Woronin (19) to be due to the presence in the host cells of a plasmodium. This disease had been so widespread and destructive in Russia, and especially in the neighborhood of St. Petersburg, that the Russian Gardeners' Association established a prize in 1872 for its scientific study. No one claimed the prize in 1873 and the competition was extended to 1875 and again to 1877.

EARLIER STUDIES

Woronin (19) took up the study of the disease in 1873 and completed his work in 1876. His results were published in a series of papers in Russian and finally in a completed form in German in Pringsheim's Jahrbucher for 1876. It is this latter paper which is generally known to the scientific world.

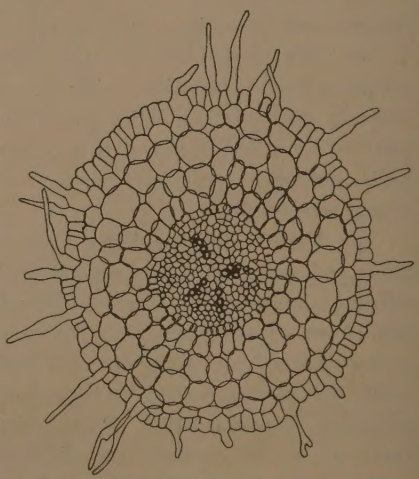

Fig. 1. Cross-section of a very young, healthy, cabbage root. (After Woronin).

A number of observations were made on the effect of the disease on the cabbage plant and the resistance of various species, but the larger part of the discussion is devoted to the structure of the normal and diseased cabbage roots and to the effect which the fungus produces upon their cells. Woronin gives Caspary the credit for the first investigation of the swollen roots. This observer was unable to find either fungus threads or bacteria in

root sections and concluded that the disease was the result of the tendency of the cabbage plants to form "sports." This view was further confirmed when he planted seeds from affected plants and the disease appeared on the seedlings. As Woronin points out, however, this planting was undoubtedly done in beds already infected by the club-root spores.

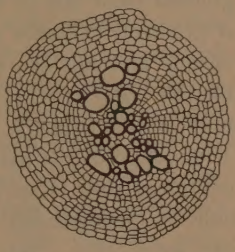

Fig. 2. Cross-section of a young, healthy, cabbage root. (After Woronin).

The difference between cross-sections from a healthy root (Text Figs. 1 and 2) and those from a diseased one (Text Fig. 3) lies in the presence in the greatly hypertrophied cells of the cortical parenchyma, of an opaque, colorless, finely granular plasma. The cortex is greatly increased in size and this enlargement is due to an increase in the number of the cells as well as to the hypertrophy of the individuals. The vessels are often bent and shoved out of their proper position in diseased roots, but, as in normal ones, only contain air. The hypertrophied cells become filled in the older roots with the small round spores of the organism to which Woronin gave the name of *Plasmodiophora Brassicae*.

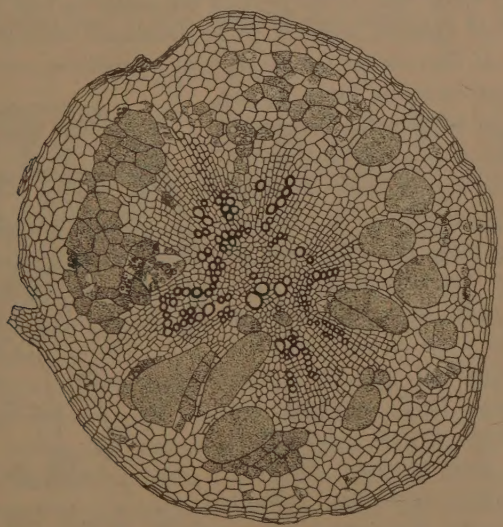

Fig. 3. Cross-section of a root badly infected with Plasmodiophora Brassicae. (After Woronin).

The plasmodium of Plasmodiophora is similar to that of other myxomycetes in that it is a jelly-like, amorphous substance in which

are imbedded granules and small oil droplets (Text Fig. 4). In the first stages of infection (Text Fig. 5), the protoplasm is so pale and colorless that it can be hardly distinguished from that of the cabbage, but it gradually becomes more and more opaque and fills the entire lumen of the cell. In cut cells, it may flow out in the form of spheres of varying size. The motion of the plasmodium in the uninjured cells is very slow and can only be distinguished by watching it for some time. Starch grains are often imbedded in the plasmodium upon which it seems to feed. After spore-formation the plasmodium usually fills the entire cell lumen (Text Fig. 3).

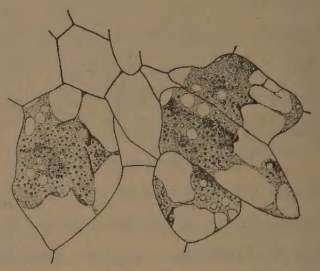

Fig. 4. Parenchyma cells from a clubbed cabbage root. (After Woronin).

In spore-formation, small round vacuoles appear in the protoplasm at some distance from each other, which make a fine network of the plasmodium. Between these vacuoles the substance of the plasmodium gathers into small, regular masses which form the spores. These seem to be bound together by some sort of a clear, slimy substance which holds them in the form of the enclosing host cells. The spores are held together, however, by no membrane other than the cellulose walls of the crucifer and no trace of a capillitum nor of a peridium such as occurs in other myxomycetes is to be found. The spore formation occurs gradually and at the same time the diseased roots begin to rot, especially if they are in wet soil. The rotting mass of such roots is composed of the broken down host cells filled with the spores of the fungus. The walls of these cells are gradually dissolved and the rotten mass contains finally nothing but Plasmodiophora spores. The spores are small, their greatest diameter never being over .0016 mm. Almost without exception they are spherical in shape, although occasionally biscuit shaped and double forms are seen. The diseased roots which are allowed to rot in the ground spread this multitude of spores throughout

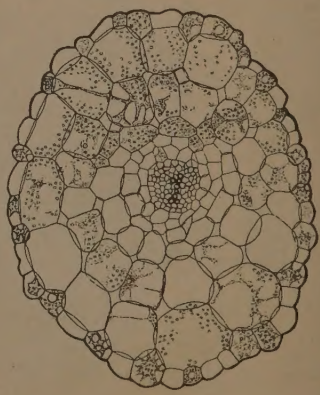

Fig. 5. Cross-section of a young cabbage root artificially infected with club-root. (After Woronin).

the soil, thus affording an opportunity for the infection of plants grown during the following years.

The spores now germinate to produce myxamebae (Text Fig. 6). These are long, spindle-shaped bodies, at one end of which is a rather long cilium. A slowly pulsating vacuole and some small granules appear in the naked protoplasmic body. The movement is produced by means of this cilium and also by a sort of creeping brought about by pseudopods similar to that of the ordinary ameba. The latter method of locomotion only appears after the ameba are a few days old.

Woronin was unable to find any of the myxamebae actually pressing their way into the host cells but was certain that their entry occurred either through the hairs or epidermis. The youngest stage he was able to observe (Text Fig. 5) was in cross-sections of young roots whose epidermal cells were infected by a plasmodium which resembled that of Plasmodiophora. This infection may occur not only in very young plants but in mature ones where the primary cortical parenchyma is already cut off. Once in the parenchyma cells of the root, the myxamebae increase with the cell content, pass over from one cell into another and call forth a very strong stimulus in the whole tissue. The life cycle just described is again repeated.

The methods wherewith this disease may be combatted are discussed in another part of this study of club-root[1] and so will not be

Fig. 6. Germination of the spores and development of the myxamebae of *Plasmodiophora Brassicae*. (After Woronin).

repeated here. It is interesting to note, however, that Woronin called attention to similar growths on the roots of the alder and of the legumes, although he did not carefully investigate them. The former he believed to be due to a fungus, while the latter he suspected to be of bacterial origin.

Eycleshymer's paper (1) was largely devoted to a confirmation of Woronin's results and a study of the spread of the disease throughout the United States. He was able to germinate the spores, apparently in quantity, and distinguished a nucleus in each of the larger forms which appeared in culture slide 4-5 days after the swarm cells had

[1] Studies on Club-Root. II. Relative disease resistance of various crucifers to club-root and methods for combatting the disease. Vt. Sta. Bul. 177 (1913), to be issued Dec. 1913.

almost entirely disappeared. He also observed in sections of tissue containing plasmodia minute bodies undergoing vibratory movements, which so resembled micrococci that he was almost convinced they were such. He suggests that while *Plasmodiophora Brassicae* is the principal cause of club-root "it is by no means improbable that bacterial forms play quite an important part."

Wakker (17) classified Plasmodiophora as one of the hypertrophytes. In these plants the influence is always chemical as well as of an indirect anatomical nature. The principal changes, due to the parasite, observed by him are:—

1. A noticeable swelling of the affected parts caused by the increase of the cortical cells.
2. Either an abnormally strong or else an irregular activity of the cambium.
3. A partial suppression or irregular form of the vessels.
4. A piling up of transitory starch.

With the exception of the minor contributions by Wakker and Eycleshymer no further study of the structure of *Plasmodiophora Brassicae* was made until, in 1893, Nawaschin (6), working on material sent by Woronin, began to review its cytology. In the meantime this branch of biology had been making rapid progress and the modern methods of fixation and staining were being worked out. It was not until 1897 that he was able to fix and stain successfully the plasmodium in the cells of the cabbage. His results were not published until 1899. Thanks to the use of Flemming's fixing solution and three color stain, he was able to present the first account of the finer structure of the parasite.

Nawaschin found the fixation with osmic acid to be particularly helpful in distinguishing the very early stages of the parasite in the host cells. The abundant fat droplets which the plasmodium enclosed made it very much darker in color than the surrounding host protoplasm. The nucleus, stained red by the three color stain, showed among these small droplets. It was never possible for him to see the actual fusion of the amebae in the host cells. The line of division between the individuals, he believed, could be partly distinguished by means of the host starch grains lying between them.

Nawaschin, while not definitely so stating, conveys the impression that he is doubtful whether the amebae actually remain long separate as individuals. The amebae themselves have no shape, save that impressed upon them by their enclosing host cell walls, and when released from this pressure they assume a spherical form. A similar

phenomenon may occur if the amount of protoplasm in the host cells is small, the parasite assuming this shape in the centre of the cell while the protoplasm is crowded into the corners. An ectoplasm and endoplasm such as often occur in amebae, seem to be lacking and the structure of the protoplasm of the Plasmodiophora is either granular or like a net. The structure of the very young amebae is difficult to differentiate as its body seems almost to fuse with that of the host. The fat occurs as droplets in the alveoli of the net-like protoplasm. These droplets contain, however, he believes, some small protein bodies which stain with haematoxylin even after the color due to the osmic acid has been removed. These bodies are not to be considered independent organs but merely as the contents of the protoplasm, perhaps even as its ground substance.

Few of the amebae contain less than four nuclei. The youngest individuals he is able to differentiate contained two. The nuclei are either spherical or egg shaped, with a distinct nuclear membrane, and enclose a nucleolus, usually at their centre. The chromatin network of the nucleus stains very faintly and can only be seen by the most careful focussing and lighting. It is stained a faint blue with gentian violet. A colorless court surrounds the densely stained nucleolus, called by Nawaschin, a "Binnenkoerperchen." His justification for the use of this latter term lies in its very peculiar behavior during nuclear division. In this process it appears to be not simply a passive protein-like inclusion of the nucleus, but an organ to which an active role is assigned.

Increase in the size of the plasmodium is accompanied by nuclear divisions, the latter occurring simultaneously in one ameba. He was never able to observe the passage of the parasite from one host cell to another by means of perforations in the walls and is of the opinion that this can not occur during the secondary growth of the root, but only in the primary tissues during the younger stages of the development of the swellings on the roots. He did not investigate this point, however. He believed that the amebae increased in numbers by a process of budding, the short pseudopodia being cut off as new individuals.

Nawaschin believed that just before spore-formation the amebae flow together to form a true plasmodium, the plasma membrane separating them being at this time broken down and absorbed. At the same time the nuclei undergo marked changes, the dense, brilliantly staining bodies in their centres disappearing while small granules fill the nuclear cavity. Vacuoles of all sizes cut up the protoplasm and

between these the nuclear divisions appear. The cytoplasm becomes granular and the nuclei, no longer densely stained, are difficult to distinguish therein. Fibrillae radiate out from these cytoplasmic granules and the nuclei have often apparently disappeared. He was doubtful, however, as to whether they had entirely disappeared or whether their presence was masked by the dense cytoplasm.

The nuclear divisions are very similar to those occurring of higher plants. A centrosome-like body from which radiates fibers seems to be present at either pole during metaphase. The bits of protoplasm become separated from each other by means of the vacuoles and round up to form the spores. No true wall or peridium seems to enclose and to hold them together.

Nawaschin was of the opinion that for a long time a symbiotic relationship exists between the cells of the host and the plasmodium living therein. The Plasmodiophora lives only in the vacuoles and apparently does not disturb the normal life functions of these cells. Even the nuclear divisions seem to occur as normally as though the intruder were not there. During divisions of the host cells, the plasmodium is carried into the daughter cells, infecting them. In this way the groups of diseased cells seen in roots are formed. The surrounding uninfected tissue remains apparently indifferent to the close proximity of the plasmodium. With the increase of the amebae, the hypertrophy of the host cells becomes more marked and the life functions of the latter become so hemmed in that, just before spore-formation in the parasite, the host nuclei and cytoplasm are crowded to the walls. The nucleolus of the host nucleus becomes enlarged as it degenerates and numerous erythrophilous granules appear in the nuclear network. In strongly hypertrophied cells, the nucleus becomes still larger though poorer in contents, until, finally, the chromatin seems entirely to disappear. In the course of its degeneration, it often puts out pseudopodia-like processes.

In an appendix to the paper just reviewed the author describes the nuclear divisions in the vegetative amebae. This process was an indirect one. The equatorial plate formed around the nucleolus split in two and the two parts moved to the poles, the spindle being entirely intranuclear. The remaining material contained in the nucleolus divided at the same time, and from it two daughter nucleoli were formed.

Two distinct types of nuclear divisions therefore occur:

1. A modified form of indirect division in the vegetative nuclei of the plasmodium.

2. Typical mitotic divisions just before spore-formation.

The writer has been unable to secure either Nawaschin's later paper (1901) on dimorphism in the cell nuclei of Plasmodiophora (7), or a review thereof; but it is presumably an elaboration of the observations made in its earlier publication.

The unusual phenomena involved in nuclear division, as described by Nawaschin, induced Prowazek (12) yet further to pursue this phase of the subject. His observations were made on sections from material fixed in either sublimate-alcohol, Perenysch's, Flemming's or Hermann's solutions and stained with Giema's azureosin, Grenacher's haematoxylin or Heidenhain's iron haematoxylin with a counter stain of Bordeaux red or alum-carmine. Both Flemming's or Hermann's solutions were found to be satisfactory preserving materials.

Prowazek's description of the resting nucleus is essentially the same as that of Nawaschin. He is convinced also that the intensely stained body called a karyosome in the nucleus is composed of two constituents:—the chromatin and a more achromatic substance. In vegetative divisions the chromatic substance spreads out on the alveolar achromatic network into fine particles. The network assumes various forms and at times the achromatic part of this body apparently disappears. The chromatic granules arrange themselves into an equitorial plate around the lighter colored achromatic portion. These granules divide, move to the poles, surround the achromatic portion again and form two daughter nuclei with similar central bodies.

In the light of the latter researches on the protozoa, the nucleus may be divided into two parts:—a somatic macronucleus which functions in the division of the chromatin and a sexual micronucleus in which is stored up the generative chromatin.

The karyosome in the divisions preceding spore-formation either retains its identity for some time and finally arranges itself in long strands and loops throughout the nucleus before finally entirely disappearing, or opens out into a number of granules on connecting fibers. The number of chromosomes in either case is apparently eight. In these latter divisions there appears at either pole of the somewhat elongated nucleus a centrosome, from which radiate delicate fibers, and the cytoplasm becomes highly granular, due to the extrusion into it of finely divided bits of chromatin. The spindle is not markedly different from that present in other ciliates being intranuclear with a minute centrosome at either pole. The chromosomes are drawn to the poles and the remains of the old central spindle, often bent and twisted, are left to connect the two groups. In spore-formation, the alveolar,

darkly stained protoplasm collects around the nuclei. These masses are in the beginning long and sickle-shaped, but later round themselves up.

Prowazek distinguishes the two sorts of vegetative mitoses as described above, but these are followed in the globular spores by nuclear divisions which he believes to represent the reduction phenomena. The nucleus divides to form one or more reduction bodies, the karyosome seems to have disappeared and the chromatin lies close to the nuclear wall. The size of the nuclei at this time is very small and observations are correspondingly difficult. The number of reductive bodies does not seem to be constant, in most cases two and in others only one. Two of these nuclei fuse at once to form a synkaryon, the centrosomes apparently fusing at the same time. The mature spore, therefore, is uninucleated. Prowazek was unable to germinate the spores. Some observations are added on the effect of the parasite on the nuclei and cells of the host. He found the nucleus of the infected cells capable of division for a longer time than the cytoplasm resulting in some binucleated cells. The effect of the fungus at first is to induce hyperplasie; later, the stimulus calls forth an increase in the size of the cells. The nucleus of the host forms occasionally around a small nucleolus, a small nucleus inside the old one, a second nuclear membrane arising inside the first. He regards this as an indication that the original nuclear membrane is a product of the nucleus and not of the cytoplasm. The nucleus becomes wrinkled and irregular in shape and finally undergoes, together with the small amount of protoplasm lining the walls, a complete degeneration. The cell sap, he finds from staining reactions, tends to become somewhat more alkaline as a result of the presence of the parasite, although dark, acid-reacting substances accumulate on the fungus protoplasts.

Some mention must be made of the work of Harper (2), Jahn (3), and of Olive (8) on the other myxomycetes; of the observations of Lister (4), Rosen (14) and of Strasburger (16) touching mitosis in these plants. Harper (2) gave the first detailed account of their nuclear division and spore-formation. His studies were made on *Fuligo varians*. In this species the nuclei divide karyokinetically, the division figures not being essentially different from those which occur in the higher plants. The spindle is sharp-pointed without a centrosome at the poles and the number of chromosomes on the equatorial plate is apparently twelve. The nucleolus disappears early. The spores are uninucleated and are formed by a process which he terms "pro-

gressive cleavage." Furrows from the surface cut into the rather dense protoplasm, segmenting it into pieces of irregular sizes and shapes and this process is repeated until each of the bits of protoplasm contain but a single nucleus.

Jahn (3) has studied various stages in the myxomycete life history including the reduction divisions. He finds it to occur in Ceratiomyxa just after the stalk is fully formed. He has also studied the divisions in which the eight swarm spores are formed. The fusion nucleus contains sixteen chromosomes while the reduction divisions show only eight.

Olive (8) found that in Ceratiomyxa the reduction divisions occur just before the spores are formed. The fusion of nuclei in pairs must occur therefore at some previous stage in the life history of the organism, although this suppositon was not confirmed by observations.

Pinoy's (10) interesting account of the part taken by bacteria in the development of the hypertrophied structures in the cabbage has not been available and the following notes are derived from Smith's (15) very complete review. Some of the cells invaded by the parasite also contained masses of coccobacilli, either singly or in pairs. Material taken by a sterile needle from the interior of large tumors produced great numbers of colonies on plates of the ordinary media. Sterile pieces of healthy turnip placed in flamed tubes were sowed with the spores of the parasite and then sealed. Colonies of aerobic bacteria appeared on the surface but ceased to grow after the oxygen was exhausted, although after five days the cells of the turnip contained various stages of Plasmodiophora. In tubes plugged with cotton, these aerobic bacteria induce the decay of the turnip. Aerobes seem to be necessary to the life of the fungus outside the host, the spores germinating only in their presence and their activities seem the primary cause of the decay of the hypertrophied roots of the cabbage.

Maire and Tison (5), the latest workers on this group, have studied in detail the cytology of *Sorosphaera Veronicae* and have made observations on *Plasmodiophora Brassicae* and a number of other forms belonging to the Plasmodiophora family. In *Sorosphaera Veronicae* they were able to observe two types of division, designated as schizogonic and sporogonic. The first type is really a double division, a mitosis of the idiochromatin and simultaneously an amitosis of the trophochromatin. The sporogonic type occurs in two stages; two successive divisions, a heterotypic and homotypic. No fusion of nuclei occurs in the spores.

These facts, it will be observed, are closely parallel with those described for *Plasmodiophora Brassicae* which these authors have studied. They disagree with Prowazek, however, as to division and fusion of the nuclei in the spores. After the spore is formed and becomes uninucleate, it remains permanently so. The number of chromosomes observed in the two sporogonic divisions was always eight. In *Sorosphaera Veronicae* there are sixteen chromosomes in the schizogonic divisions and in the first of the sporogonic, while the second of these only show eight.

The Plasmodiophoras, these authors believe, ought to be considered as a group intermediate between the sporozoa and the myxomycetes and descended more or less directly from the flagellates.

THE PRESENT STUDY

METHODS

In the present study of *Plasmodiophora Brassicae* and its relation to its host, the material was taken largely from cabbage plants grown in cold frames. Sections from fresh plants were examined and pieces were fixed in chrom-acetic and Flemming's weaker and medium solutions. The latter two were found to be the most satisfactory on the whole, although considerable shrinkage was produced by all the preserving fluids tried. Mixtures containing a much higher percentage of acetic acid were employed but the results obtained were no better. The protoplasm in the cells of the hypertrophied roots seems to be very sensitive to fixing solutions and no combination thus far used by the writer preserves them without some plasmolysis. Staining was secured either by Heidenhain's iron haematoxylin or by Flemming's triple stain. The latter was found to afford the better results and the haematoxylin sections were used only for purposes of comparison. Bleaching with hydrogen peroxide was always necessary before staining, but unbleached sections from material fixed in Flemming's showed the distribution of fat in various portions of the plasmodium very favorably.

Observations were made with a two mm. Zeiss apochromatic with the six ocular, but the drawings were partly made with combinations of other higher power oculars with the same objective.

SECTIONS OF FRESH MATERIAL

Woronin (19) has already so carefully noted the structures visible in sections of unfixed material cut with a razor and mounted in water,

that little can be added. The constant association of bacteria with the myxomycete does not seem to have impressed him as it did Pinoy (11). These bacteria can always be seen in active motion inside the myxomycete cysts. When these walls are broken the enclosed material flows out along with the bacteria. The latter are then seen to be cocci of about a micron in diameter, moving either singly or in twos. Cultures in media from these organisms were all unsuccessful, but as they are always found in combination with the myxomycete, they must play some important role in the life history of the parasite.

THE RELATION OF PLASMODIOPHORA TO THE HOST

1. *Method of infection.* No attempt has been made in the present work to ascertain the manner in which the parasite makes its entrance into the cells of the crucifer. Woronin (19) has undoubtedly described and figured about as early stages as are recognizable. The colorless ameba without permanent shape would be difficult to differentiate from the protoplasm of the host. Doubtless the infection must occur either through the epidermis or the root-hairs. As has been first shown by Marshall Ward (18) the nodule-producing organisms or legumes enter their roots by penetrating the root-hairs in the form of an infection thread. The organism then pushes its way into the deeper tissues in thick columns from these primary infections. Woronin's figure (Text Fig. 5) of early infections would indicate that the entrance may be made through the walls of any of the epidermal cells, since the plasmodium seems to occur in the majority of them. In the light of Pinoy's (11) researches on the invariable symbiotic relationship of bacteria and myxomycete, the early stages might throw some light on the relative parasitism of the two. It may be entirely possible that the original parasite is the bacterium and that the myxomycete, while it may be of service in breaking down the cell walls, is only a scavenger which lives either on the by-products of the bacteria or on the bodies of the bacteria themselves.

2. *Spread of the fungus in the tissue.* As it is not possible to see the forward end of the plasmodium in its progress into new cells, we can only conjecture the method of its spread from the grouping and present condition of the infected cells. These cells usually occur both in cross and longitudinal sections as very distinct groups (Plate I, Text Fig. 3). Such groups might be formed in at least two ways: (1) by the division of an infected cell carrying the plasmodium into a series of daughter cells; (2) by the penetration of the walls and infec-

DESCRIPTION OF PLATES

All figures were drawn by the aid of an Abbe camera lucida. A Zeiss 2 mm. apochromatic objective in combination with various oculars, Nos. 2, 4, 6 and 12, was used for the outlines, and the details were always added by means of the No. 6 ocular. The approximate magnification is given in each case.

Fig. 1. Longitudinal section, showing the spread of the Plasmodiophora up and down the root. × 850.

Figs. 2-7. The Plasmodiophora in the host cells. The relation of the myxomycete to the host nucleus and starch will be noted. The connecting strand of myxomycete protoplasm through the host cell walls will be seen in Nos. 2, 3 and 5. × 1200.

Fig. 8. Cross-section of a root fixed in Flemming's solution, showing the distribution of fat. The entire root was affected by Plasmodiophora. × 5.

Fig. 9. Three cells from the same root; the cell at the left from immediately under the epidermis, the others from deeper parenchymia. × 450.

Fig. 10. Nuclear division in a diseased cell. The nucleus has been pushed to one side of the cell. × 1200.

Fig. 11. Nuclear division in a cell not containing the organism, but surrounded by infected cells. × 1200.

Figs. 12, 13 and 14. Cell division in infected cells. The completion of the cell wall earlier on one side than on the other will be noted. The myxomycete retards its extension. In Fig. 12 the remains of the barrel-shaped central spindle still remains on the side affected by the parasite. × 1200.

Fig. 15. Two cells in early stages of infection. × 1200.

Fig. 16. Host cell containing myxomycete spores. × 1200.

Figs. 17, 18 and 19. Normal cabbage nuclei from uninfected cells. × 1200.

Figs. 20 and 21. First indications of degeneration of nuclei from infected cells. × 1200.

Fig. 23. Degenerating nucleus with the chromatin network broken down. × 1200.

Figs. 24 and 25. Apparently abnormal divisions in host nuclei. The spireme seems to be formed largely in the nucleolus. × 1200.

Figs. 26 and 27. Common stages in host nuclei degeneration. The supplementary nucleoli surrounded by a clear court are the organs described by some authors as new nuclei inside the old one. The nucleus has lost its smooth contour in outline. From cells containing Plasmodiophora spores. × 1200.

Figs. 28 and 29. Last stages in degenerations of host nuclei. From cells filled with spores. The contents are collected at the periphery in irregular strands and clumps. × 1200.

Figs. 30, 32, 33 and 34. Stages in spore formation in the plasmodium. The collection of cytoplasm around the nuclei are shown in Figs. 30 and 33.

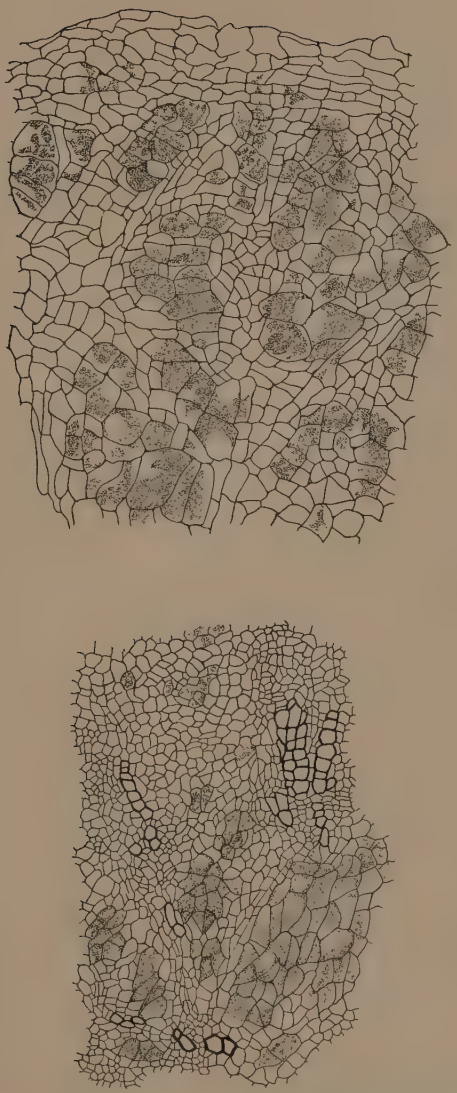

PLATE I. Portion of cross-section of root badly affected by Plasmodiophora. Spore-formation has not yet begun in the plasmodium. Upper figure from the cortex, lower from the central cylinder.

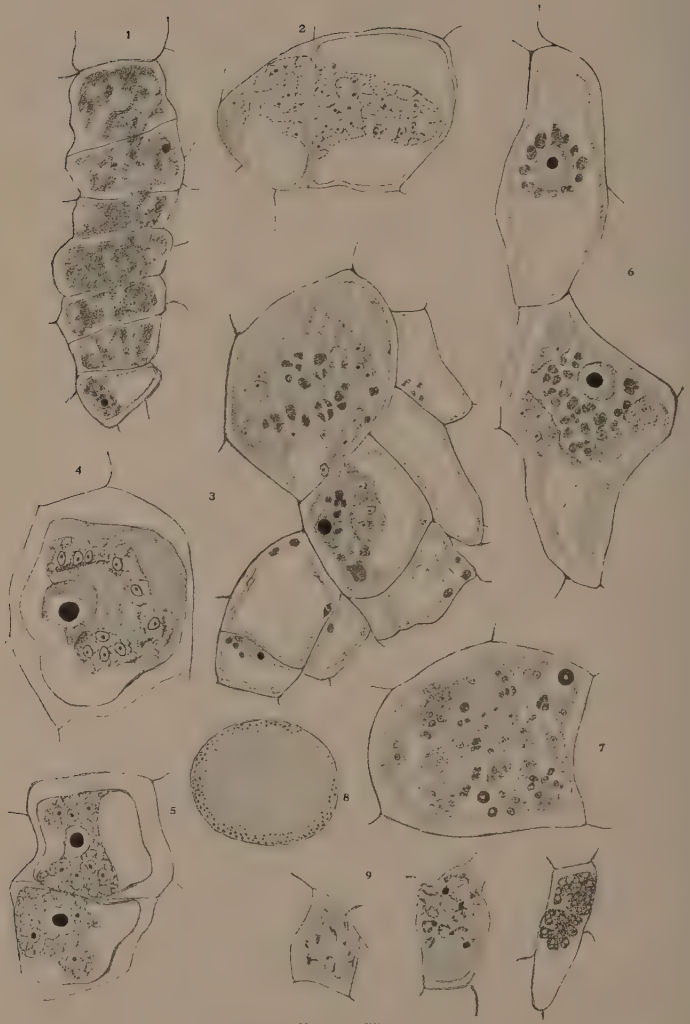

PLATE II.

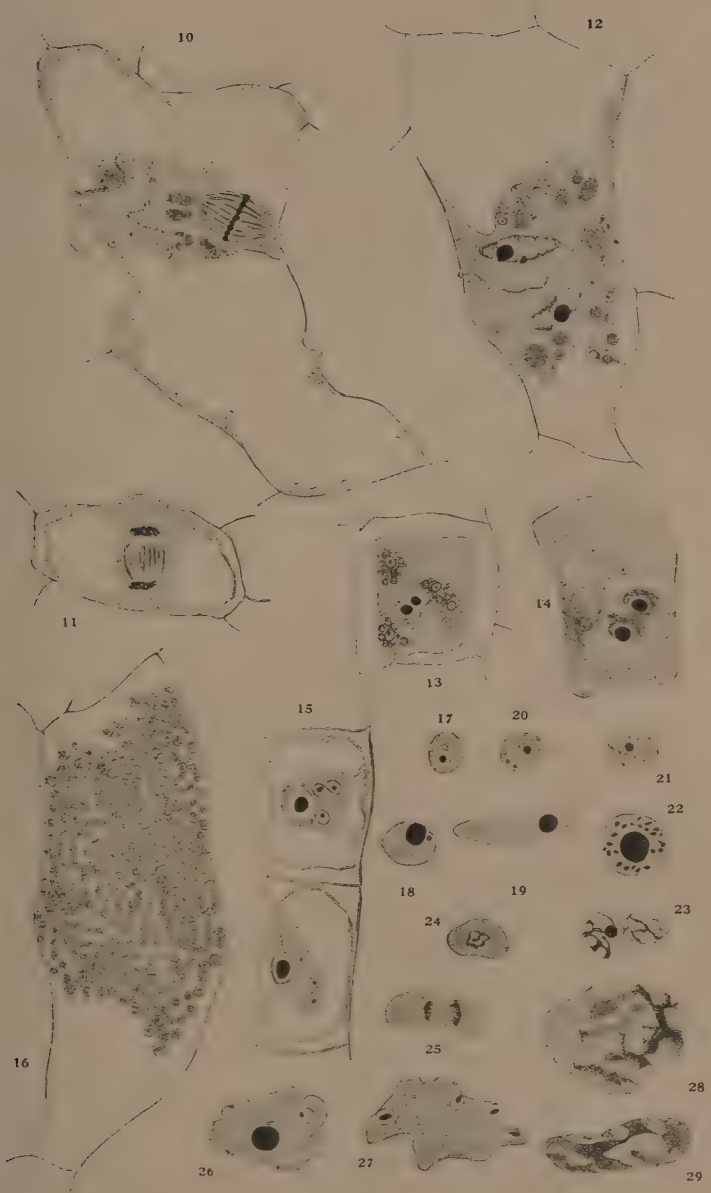

PLATE III.

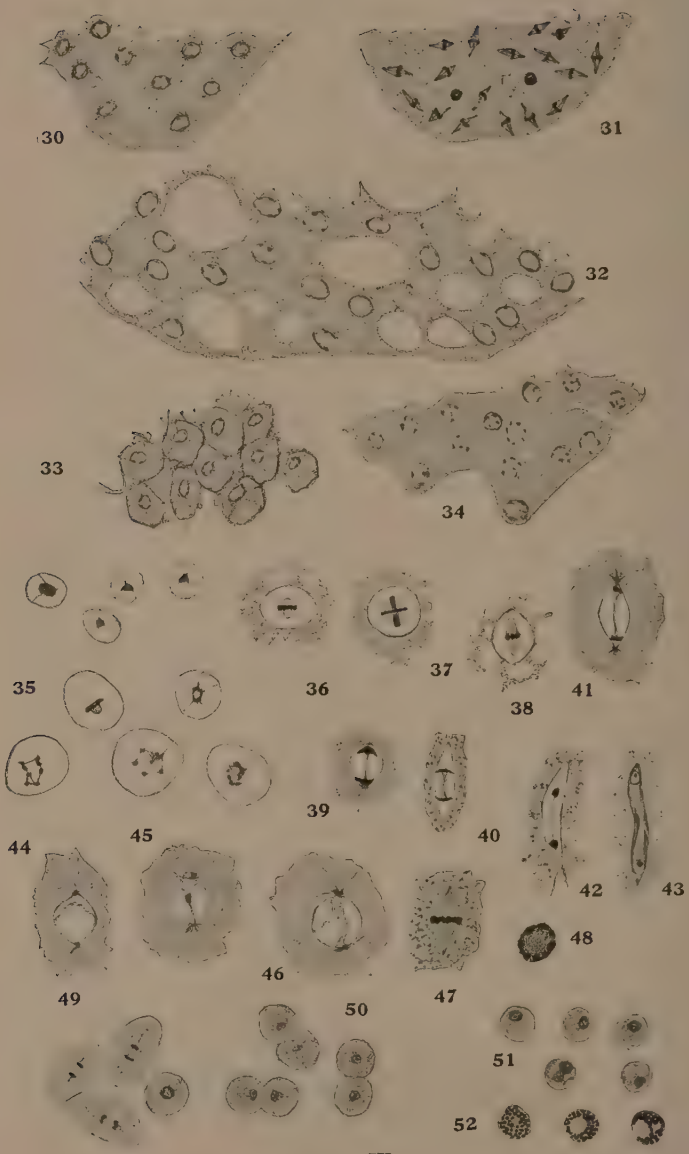

PLATE IV.

The formation of large vacuoles is seen in Fig. 32. Fig. 34 shows edge of plasmodium about ready to form spores. × 1200.

Fig. 31. Nuclear divisions preceding spore-formation. × 1200.

Fig. 35. A comparison of the prophase in the vegetative divisions and the first method of dissolution of the central body in the divisions to form the spores. The upper six nuclei are stages in vegetative prophases and the lower three spore-forming prophases. The collection of chromatin on the outside of the central body is well seen in nucleus on the middle right. × 2100.

Figs. 36 and 37. Early stages in the formation of the spindle. The remains of the central body can be seen in Fig. 37. × 1200.

Fig. 38. Metaphase in vegetative nuclear division.

Figs. 39, 43. Later stages in nuclear division. The reconstitution of the nuclei is shown in Figs. 42 and 43. × 2100.

Figs. 44-46. Spiremes in nuclear divisions preceding spore-formation. × 2100.

Fig. 47. Metaphase. × 2100.

Fig. 48. Metaphase, end view. × 2100.

Fig. 49. Spores already cut out but containing nuclear divisions. × 2100.

Fig. 50. Cell division in such spores. × 2100.

Fig. 51. Mature spores showing nuclei and vacuoles. × 2100.

Fig. 52. Mature spores showing fat droplets. × 2100.

tion of a row of neighboring cells. That the first method is the one of frequent occurrence is well evidenced by figures 10, 12, 13 and 14. In these cells the actual partition of the plasmodium by means of the growing cell wall can be seen. This is unnecessary in many instances for, as Nawaschin (6) pointed out, the plasmodium stretches out into long pseudopodia which seem to break off to form new individuals. Some of these fragments might be included in each of the new cells if their position happened to be such that the cell wall was placed between them. Fig. 15 shows two cells, evidently sister cells, each infected by the fungus. The second method of infection, by actual penetration of the walls of undiseased cells, is more difficult to demonstrate. Figures 2, 3 and 5 would indicate that it could occur. The very small hole in the host cell wall may be readily overlooked even in sections with too dark a stain for nuclear or cytoplasmic details. Like other parasitic species this fungus wastes no energy in making an unnecessarily large hole. The possibility that these connecting strands of plasmodium might be due to an incomplete division of the parasite when the host mother cell divided, seems to the writer to be a rather remote one. If the cell wall is built in, with sufficient energy to separate the plasmodium so nearly, the small remaining strand would not be spared.

3. *Relation of the parasite to cell and nuclear divisions.* Nawaschin and Prowazek do not seem to have studied this phase of the question in detail. Prowazek (12) states, however, that the nuclear divisions occur after cell divisions have ceased and that binucleated cells occur as a result. The nuclear divisions, as the writer has found them (Figs. 10 and 11), occur normally in most cases even in cells badly infected with the plasmodium. Abnormal divisions, such as those shown in Figs. 24 and 25, are also to be found. The process seems to be a modified form of amitosis, in which the spireme is formed largely from the nucleus and the remaining portion of the nucleus has only a passive role. These cases are rare, however, and, so far as can be seen (1) the process of mitosis is entirely regular and normal. Cell division, as Prowazek has observed, is retarded by the presence of the foreign organism in the cells. Figures 12, 13 and 14 show the relation of the growing cell wall to the Plasmodiophora. The cell wall is not completed symetrically on the two sides of the cell. On the side unoccupied by the parasite the wall is completed while, as strikingly shown in figures 12 and 14, the plasmodium has prevented its extension to the other wall. The remains of the central barrel-

shaped spindle are still to be seen in figure 12. No binucleated or multinucleated cells were observed.

4. *Relation of the parasite to storage starch.* The hypertrophied cells of the diseased tissue contain considerable quantities of cabbage starch often grouped around the nucleus. Reed (13) has already noted this fact. In cells that are filled with the plasmodium, this starch lies imbedded among its folds (Figs. 3, 6 and 7). This is true, however, only of the younger stages of the plasmodium. At the time of spore-formation, the starch has almost entirely disappeared, although a few grains can sometimes be seen scattered among the spores. The inference naturally to be drawn is that the starch is consumed in the nutrition of the parasite, but no evidence as to enzymic action on the grains was observed.

5. *Effect on the host nuclei.* The normal cabbage nuclei contain one large and sometimes one or more additional small nucleoli (Figs. 17, 18 and 19) in a finely reticulated nuclear network. The first evidence of abnormal conditions due to the presence of Plasmodiophora is an increase in the number of nucleoli (Figs. 20, 21 and 22)and this change does not occur until the myxomycete is approaching spore-formation. The number of these supplementary nucleoli is variable and their form is either that of a sphere or of a drawn-out droplet. They sometimes lie free in the nuclear reticulum and in other cases are surrounded by a clear court, as is the large nucleolus. The nuclear membrane at this time is still filled out, so the nucleus is regular in outline. The formation of these secondary nucleoli is an interesting problem in cellular pathology. If the nucleolus is to be regarded as a store of reserve proteid material, it would seem that the amount increases during the earlier stages of the attack of the parasite. The nucleus at this time has undergone a marked hypertrophy but in the succeeding stages of degeneration (Figs. 26 and 27, drawn from cells in which the plasmodium has broken up into spores) the host nucleus has increased in size and has lost its previous regular outline. The small nucleoli at this time lie in a clear court surrounded by a distinct membrane. This has caused Prowazek (12) to claim that smaller nuclei with a distinct nuclear membrane can be formed inside the large nucleus. The writer deems this to be not an error in observation but in interpretation. The small nuclei he observed were apparently nucleoli surrounded by a clear court. The fact that the nuclei are plasmolyzed at this stage is an indication either that the osmotic strength of the solution in the nucleus is very much weakened or that that of the

cell-sap is increased. In view of the enormous increase in the size of cells in which spore-formation is occurring (Text Fig. 3) the latter hypothesis seems more probable. It may be, too, that the formation of secondary smaller nucleoli is an endeavor on the part of the nucleus at this time to maintain its osmotic equilibrium with that of the cell-sap. On the other hand these small bodies in the nucleus may be regarded merely as waste products thrown out in response to some partial poisoning by the myxomycete. The final stages in nuclear degeneration are seen in figures 23, 28 and 29. The material in the nucleus collects into irregular strands which assume a peripheral position in the irregular and hypertrophied nucleus. Such nuclei are to be found in cells where the myxomycete spores are completely formed. Paratore (9) has observed similar conditions in the degenerating nuclei of the legume nodule cells where the nucleolus disappears. Large red bodies take its place in the nucleus and in later stages the nucleus becomes ameboid with strongly colored processes. Vacuoles then appear in the nucleus and in the chromosomes.

THE STRUCTURE OF PLASMODIOPHORA BRASSICAE

The plasmodium in its early stages is very clear and resembles so closely the protoplasm of the host cells that it is almost impossible to make out its outlines. In the young stages of infection there usually appear 3-6 nuclei in the plasmodium, Fig. 15. The nuclei increase in number rather slowly at this time, if the small number of divisions is to be taken as an indication. As the myxomycete protoplasm increases in quantity it becomes denser, especially toward the tips, where it often stains so densely that it is almost impossible to differentiate the nuclei. Further back it is usually of a more open and vacuolate structure. At times the cytoplasm may become almost thready with small granules on the fibers (Fig. 2). Their staining reaction would lead one to consider these to be of a protein composition. In the tips of the pseudopodia the nuclei are small and the central body almost fills the nuclear cavity. In the older parts the nuclei are very much larger and are often drawn out into a spindle form with dense staining ends and with the central body failing to fill a considerable part of the nuclear cavity. In the earlier stages the host protoplasm fills the cell with the exception of a number of vacuoles while the older plasmodium pushes this to the wall and practically fills the cell lumen. The writer agrees with Nawaschin that the number of plasmodia is increased in the cells by a process of fragmentation. Pseudopodia are pushed out

which become attached by finer and yet more fine strands of protoplasm, until they become entirely separated from the main body of the myxomycete and form new individuals. This must be of particular importance in the spread of the parasite by means of host cell division. The actual cutting of the plasmodium into two parts by a growing cell wall may, due to this self fragmentation, be made very easy or possibly entirely superfluous.

THE STRUCTURE AND DIVISION OF THE PLASMODIOPHORA NUCLEI

Nawaschin and Prowazek were able to observe a fine, light-stained recticulum in the resting nucleus of the Plasmodiophora, inside of which was the dense central body. The writer has been unable even in very darkly stained sections to observe any such recticulum. The nuclei do show very fine fibers running from the central body, as in the upper four nuclei of figure 35, but these may only be the early stages of spireme formation.

The stage for critical study in Plasmodiophora nuclei are the two types of division found by Nawaschin and Prowazek. The features of these mitoses have been already reviewed and the process as here observed may now be described. A careful study of nuclear divisions in sections will soon convince anyone that there are two apparently distinct and different methods. The best place to find both types is in the plasmodia just as spore-formation is beginning. At this stage the plasmodium seems to be growing rapidly and vegetative divisions are frequent, while in other parts the nuclear changes preceding spore-formation are progressing. As has been observed by Harper (2) in Fuligo, and Nawaschin (6 and 7) and Prowazek (12) on Plasmodiophora, the nuclear stages are all nearly the same in one portion of the plasmodium. In the vegetative divisions this may mean that only one lobe or pseudopod has the nuclei all in metaphase, while in the divisions preceding spore-formation nuclei of the portion of the myxomycete occupying one host-cell are all in prophase. The cause for the simultaneous action by the nuclei in one portion is not clear, but it may be assumed to be due to the same nutritive conditions and possibly in spore-formation to a certain ripeness, if such a term could be applied to a myxomycete. This might imply either a definite amount of stored materials, an accumulation of waste products from which the fungus could not free itself, or that the external environment had become unfavorable to continued active growth. As *Plasmodiophora Brassicae* forms spores when the cabbage or other cruciferous plant is not more than half

grown, the latter hypothesis would be hardly tenable. A much larger number of spores could be formed if the plasmodium continued to increase in size for at least a month, as its substratum would allow it to do. The formation of a plant of definite bulk, a species of size-morphaesthesia to use the phrase coined by Noll, is not apparent as spores are formed in large and small cells. The writer is, therefore, inclined to think that readiness for spore-formation in Plasmodiophora, while it may be somewhat dependant on size, accumulated waste and on the external environment, will not occur unless there is stored an abundance of food materials for the young spores.

Spore-formation, as here observed, usually begins among the outer infected cells of the cortex while the deeper lying ones are the latest to cut up to form spores. In a section of a root where spore-formation is ready to begin on the periphery, but still contains plasmodium in its pith, the amount of fat in the various regions as shown in unbleached sections from material fixed in one of the Flemming's solutions is of interest (Fig. 8). The peripheral cells filled with myxomycete protoplasm will be seen to be black with the contained fat globules, while this diminishes rapidly centripally. In figure 9, the cell to the right is from near the periphery, the middle one is 8-10 cell-layers deeper, and the left hand one about the same distance still deeper in the root. The accumulation of this fatty substance in the form of minute droplets is seen in the three spores in figure 52. This accumulation of fat as shown in the right hand cell of figure 9, in which the nuclear divisions preceding spore-formation are about ready to begin, is necessary to the cutting up into spores.

The vegetative nuclear divisions. The four nuclei shown in the upper part of figure 35 may be considered to be preparing for division. The central body is not always perfectly spherical but it is at times drawn out into processes which make it appear somewhat angular. In very lightly stained sections the writer believes that he has been able to observe that the outer portion of this body stains darker and is apparently denser than is the centre. This outer part is composed of granules. In the next stage these granules separate—middle right nucleus of figure 35—and form a ring around the equator of the remains of the central body. The number of these granules cannot be determined on account of their small size. They arrange themselves usually around the equator of this body as in figure 37, but sometimes the ring lies more to one end of it as in the middle left nucleus of figure 35. This body often forms a whetstone shaped structure as in figure 37.

The opinion is expressed that the spindle is formed from part of the material in this body. It is very difficult to be certain that this body gives rise to the fibers, but figures such as Nos. 36 and 37 would indicate that such is the case. At first this spindle is almost globular, but it lengthens out so that the poles almost, if not entirely, touch the nuclear membrane. The nucleus now lengthens out (Figs. 38-43) and the chromosomes are found at the poles, as caps. The central body does not always appear. The chromosomes form a dense mass (Fig. 42), but later the central body can again be distinguished. The nucleus at this time is very long and often twisted and until stages such as that shown in figure 42, the masses of chromatin are attached by the shrunken remains of the old spindle (Figs. 39, 40, 41, 42).

The writer has been unable to differentiate a centrosome in this type of division. Radial fibers occasionally show at the poles (Fig. 41), but their active part in chromatin division is questionable as in many instances they do not appear at all.

The nuclear divisions preceding spore-formation. The central body disappears in these divisions and the spireme formed stains so lightly that the nuclei seem to have disappeared. The breaking up may occur as Prowazek (12) has pointed out in two ways. Maire and Tison (5) were unable to differentiate these types and it may be that intermediate stages may occur. The three lower nuclei in figure 35 illustrate the first of these methods. The central body apparently explodes into eight granules which lie further separated than they do in vegetative divisions. They are connected by fine strands. In the second method of chromosome formation, the central body seems to persist for some time on one of the turns of the spireme (Figs. 44-46). It becomes lighter staining in its reaction and finally disappears.

About this time lines radiating out from the two poles into the cytoplasm begin to appear. These grow out to some distance (Figs. 44-46), but it has not been possible to differentiate a centrosome to which they are attached. In metaphase (Figs. 47 and 48) the spindle becomes sharp-pointed and the chromosomes arranged at its equator. The fibers radiating out from the poles have disappeared at this time, and, while the ends of the spindles stain dark, there are no centrosomes. The disappearance of the radial fibers at the time when they would be of service in anchoring the spindle is peculiar. It would seem to indicate that they may only be paths for the transmission of materials for the building of the spindle and not fibers active in chromatin division. The writer has not been able to count the chromosomes with any

accuracy, but is certain that there are not more than eight. It was not possible to ascertain the number of times the nuclei divide in these mitoses preceding spore-formation, but there is considerable variation in their size as can be seen by a comparison of figures 47 and 49. He is unable to say whether either of these is a reduction division. In view of the careful distribution of the material in the central body, it would be expected that this would be time at which a reduction division would be introduced. The minuteness of the nuclei and the difficulty of counting the chromosomes makes the work very uncertain, but based upon a priori reasons alone, the reduction would be expected here. Maire and Tison (5) believe, however, that it occurs in the first of the divisions to form the spores in *Plasmodiophora Brassicae* and the writer entirely agrees with them. The number of granules on the spindle in somatic division is not possible to determine, but is probably nearer sixteen than eight. The above mentioned authors apparently were unable to count the number in *Plasmodiophora Brassicae,* but the number in *Sorosphaera Veronicae* was about sixteen. Prowazek's (12) observation of supplementary nuclei in the rounded-up spore is either due to abnormal conditions or to such errors in observation as might occur in handling such small structures. This work would put it into agreement with that of Olive (8) who found the reduction divisions immediately before spore-formation in Ceratiomyxa. The time at which the nuclear fusions occur must remain for future work, but, as Maire and Tison (5) have pointed out, they will be very difficult to find.

SPORE-FORMATION

The method of segmenting the protoplasm into spores is quite different in *Plasmodiophora Brassicae* from that described by Harper (2) for *Fuligo varians.* No dense mass of protoplasm, such as is found in the latter form, is present in Plasmodiophora to render the segmentation a difficult process.

As has been noted by the previous writers on the club-root, the host cells are almost filled with the parasite at the time of spore-formation. Large and small vacuoles appear in large numbers in the plasmodium while the cytoplasm collects around the nuclei (Figs. 30 and 32). These vacuoles fuse and cut the protoplasm into uninucleated spores (Fig. 33). Nuclear divisions may occur in some of these (Fig. 49) and the nuclei resulting from such a division each give rise to a spore by a process of constriction.

Prowazek (12) has observed, as his figure 31 shows, a similar spore-formation and it would seem to be a regular occurrence in *Sorosphaera Veronicae* to judge from the plates of Maire and Tison (5), in their figures. The spores round up and are always uninucleated (Fig. 51). The nucleus does not show a central body and is rather difficult to differentiate. One or more vacuoles of about the same size as the nucleus are always present and might be confused with it. This is the only explanation the writer can offer of Prowazek's (12) observation of two or more nuclei in the spores. Maire and Tison (12) have also been unable to find any of these supplementary nuclei. The abundance of fatty material stored up in the spores is shown in figure 52.

COMPARISON OF THE TWO METHODS OF DIVISION

The two methods of division are essentially the same. The points of divergence, as the writer makes them out, lie in the formation of the spireme and in the behavior of the central body. Both divisions are mitotic so far as the idiochromatin is concerned. In the vegetative divisions, the spireme is formed by a separation of the granules of the idiochromosomes. These granules had been previously concentrated as a hollow sphere enclosing the trophoplasm of the central body. Siedlecki, as quoted by Prowazek (12) has observed a similar condition in the karyosomes of the coccidia:—"In this form, the basichromatin forms the peripheral portion while the plastin-like substance, oxychromatin, is collected in the centre of the cell." Heidenhain in "Plasma und Zelle" quotes with apparent approval Flemming's opinion that the nucleolus is always surrounded by a thin layer of true chromatin. While the spireme here only occupies a portion of the nuclear cavity, it is a true spireme. It is hardly necessary to add that the splitting of granules on this thread (if it occurs) is too small to be seen under our present microscopes.

In the nuclear divisions before spore-formation, the material in the central body is distributed in one of the two ways described throughout the entire nucleus on a spireme thread. This body is placed on one of the strands and gradually becomes smaller and with a lighter reaction to the stain, or splits into small granules which must later become soluble before the spireme thread is completed. It would seem that the material in it must by either of these methods be reduced to something of a liquid condition before this distribution could occur. A much finer breaking up of it should result than in the vegetative

divisions, and the splitting of the ids or character-bearing portions might be more accurate.

The division of the remains of the central body in the vegetative mitoses has assumed an importance that it probably does not deserve. The writer is certain that the role of this faintly staining bit of trophoplasm is not different from that of similar structures in higher plants. In the latter it usually disappears before division, but remnants of it often are to be seen to the time of spindle-formation. No attempt is made in the nuclei of *Plasmodiophora Brassicae* to divide this body equally and in some cases it almost entirely, if not completely, disappears. Maire and Tison's (5) splitting of the vegetative nuclear divisions into an idiomitosis and a trophomitosis, while stating the facts, is really unnecessary. The essential of the division is the true mitotis of the chromatin, while the pinching into two parts of the remains of the central body is secondary.

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